regions (loops and β-barrel) of the protein. One strategy was to cleave Opa60 with trypsin to isolate the micelle-embedded barrel. Based on the lack of chemical shift perturbation observed, the β-barrel structure remained intact after cleavage. The resulting simplified spectra greatly aided the assignment of the β-barrel. Additional strategies, such as temperature variation and synthetic peptides, were required to assign the loop regions. Thus, by combining these assignment strategies, the solution NMR structure was determined and reveals that Opa60 is a canonical eight-stranded β-barrel and the HV loops are long, disordered, and highly dynamic.

922-Pos Board B691
Multiple Substrate and Domain Binding in Non-Ribosomal Peptide Synthetases
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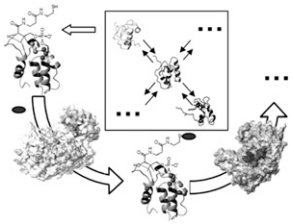
Non-ribosomal peptide synthetases (NRPSs) are enzymatic systems that synthesize important natural products in bacteria and fungi, often with pharmaceutical applications (antibiotics, antitumor agents or immunosuppressants). NRPSs use a series of domains, organized in contiguous modules, to covalently load substrates and condense those loaded on adjacent modules in an assembly line fashion. The substrates may be further modified by tailoring domains. Such multiple catalytic steps require a series of transient, sequential domain/domain and domain/substrate interactions, which are currently poorly understood. We have used NMR to probe and characterize binding sites for several NRPS domains. We show that chemical substrates do interact with NRPS domains and may be involved in modulating domain interactions. We also present the solution structures of hitherto uncharacterized domains and discuss the role of protein dynamics. We introduce novel NMR approaches necessary for such challenging systems (53 kDa domains, dynamic proteins). Understanding NRPS domain communication is a prerequisite to understand the biosynthesis of many natural products and offers prospect for reprogramming NRPS assembly lines.



923-Pos Board B692
The Study of CXCL7-CXCL12 Chemokine Heterodimer by NMR Spectroscopy
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Chemokines are small regulatory proteins that play an important role in the functioning of mammalian immune system. They are involved in wound repair, hematopoiesis, organ development, and protect organism from inflammation. However, they are also associated with serious inflammatory disorders, such as autoimmune diseases, atherosclerosis, and cancer. Recently, it became apparent that different chemokines can interact affecting the biological activities of each other. Moreover, it has been shown that the disruption of such functional interactions between chemokines can be of direct therapeutic benefit (Koenen, von Hundelshausen et al. 2009). Our mass spectrometry (MS) data shows that CXCL7 and CXCL12 form heterodimers (Carlson, Baxter et al.). To design targeted, anti-inflammatory pharmacological agents that will inhibit the interactions between CXCL7 and CXCL12, the structural model of the heterodimer is required. Here, we used NMR spectroscopy to identify the interaction interface between CXCL7 and CXCL12 chemokines. Based on these data, a molecular model of CXCL7-CXCL12 heterodimer is proposed.

924-Pos Board B693
Structure and Dynamics of Calmodulin Bound to Nitric Oxide Synthase Peptides
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Nitric oxide synthase (NOS) plays a major role in a number of key physiological and pathological processes. Knowledge of how this is regulated is important. The small acidic calcium binding protein, calmodulin (CaM), is required to fully activate the enzyme. The exact mechanism of how CaM activates NOS is not fully understood. Studies have shown CaM to act like a switch that causes a conformational change in NOS to allow for the electron transfer between the reductase and oxygenase domains through a process that is thought to be highly dynamic. To investigate the dynamic properties of CaM-NOS interactions, we determined the solution structure of CaM bound to the inducible NOS (iNOS) and endothelial NOS (eNOS) CaM-binding region peptides. In addition, we investigated the effect of CaM phopsphorylation. The tyrosine 99 (Y99) residue of CaM is reported to be phosphorylated in vivo. We have produced a phosphomimetic Y99E CaM to investigate the structural and functional effects that the phosphorylation of this residue may have on nitric oxide production. All three mammalian NOS isoforms were included in the investigation. Our results show that a phosphomimetic Y99E CaM significantly reduces maximal synthase activity of eNOS by 40 % while having little effect on nNOS and iNOS activity. A comparative NMR study between phosphomimetic Y99E CaM and wild type CaM bound to the eNOS CaM-binding region peptide was performed. NMR relaxation experiments were then performed to monitor the intramolecular dynamics of the CaM-NOS complexes. This investigation provides important insights into how the increased electronegativity of a phosphorylated CaM protein affects the binding, dynamics and activation of the NOS enzymes.

925-Pos Board B694
77Se NMR of Selenoproteins
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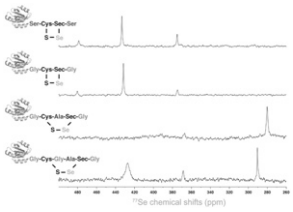
Selenoproteins are a specialized group of enzymes that contain the rare amino acid selenocysteine (Sec). The majority of selenoproteins are oxidoreductases in which the reactive Sec forms a selenylsulfide bond with a partner cysteine, positioned up to two amino acids away. It is predicted that the nature of the residues forming this redox motif and the size of the resulting ring control Sec's reactivity. We demonstrate that it is possible to probe Sec's local environment and flexibility in these redox motifs using 77Se NMR spectroscopy. We are able to record the 77Se chemical shifts and relaxation properties of proteins with representative redox motifs as a function of increasing ring size at the oxidized state. Theoretical calculations of the chemical shielding tensor are used to interpret the conformational preferences in these redox motifs. We discuss how the local environment fine tunes Sec's reactivity.



926-Pos Board B695
Site-Resolved Measurements of Hydration Dynamics in Hen Egg-White Lysozyme
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Solution NMR spectroscopy of proteins is dramatically limited by the size, stability, and solubility of the proteins of interest. Encapsulation of proteins in the protective nanoscale aqueous interior of reverse micelles (RM) alleviates these limitations by allowing larger proteins to tumble faster in less viscous, hydrophobic solutions. In addition to these benefits, RM encapsulation slows the dynamics of the surrounding water, reduces hydrogen exchange, and eliminates bulk water interactions with the protein. As previously demonstrated with the 8.5 kDa protein ubiquitin, this allows for the study of interactions between biological macromolecules and water which are important for the structure and function of proteins and traditionally have been difficult to measure experimentally. This method has now been applied to the 14.4 kDa enzyme hen egg-white lysozyme (HEWL) and the hydration dynamics of the system are described. Supported by NSF grant MCB 0842814 and NIH postdoctoral fellowship GM087099 to N.V.N.

927-Pos Board B696
Physical Properties of a Calmodulin Hydrogel as a Scaffold for Protein Immobilization
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Current approaches in the study of protein function following immobilization on solid supports do not address how the properties of the support material affect protein structure and dynamics. In order to more efficiently create appropriate immobilization systems, general principles regarding the interaction between the material properties and broad classes of proteins need to be understood. Our goal is to gain some insight as to what material properties are